The Effects of the N-Terminus of β-III-Spectrin on Actin Binding

Anna K. Andrick and David D. Thomas
University of Minnesota College of Science and Engineering

Abstract

In previous experiments it was shown that when the N-terminus of β-III-spectrin, a ~50 residue disordered region, was truncated β-III-spectrin lost all binding affinity to actin[1]. We performed binding studies in the form of actin co-sedimentation experiments to determine if the N-terminus of β-III-spectrin alone in the form of a synthetic peptide binds to actin and, if so, how much it contributed to the actin-spectrin interaction. We found that the N-terminus does bind to actin; however, a consistent K_d has yet to be determined. The co-sedimentation experiments were systemically adjusted in an attempt to improve the consistency of the calculated K_d. The major attempts at improving the results were increasing the starting concentration of the N-terminal peptide for each sample, incorporating use of a standard curve using different concentrations of the N-terminal peptide to determine the percent bound to actin, and preparing F-actin the same day as the experiment. Another way to check if the N-terminus does indeed contribute to the β-III-spectrin/actin interaction is to use EPR (electron paramagnetic resonance) to determine the conformation of the N-terminus in the presence of actin. To perform this experiment new cysteines must be mutated into the N-terminus sequence while other naturally occurring cysteines must be mutated out. In this way, the spin labels only attach to the N-terminus and not the ABD. These mutations are still in progress. To better understand possible structures of the N-terminus in relation to binding, we began using the computer simulation program NAMD (Nanoscale Molecular Dynamics). Thus far, the N-terminus has shown that helical structures are possible even without actin.

Methods

Actin Co-sedimentation *– For the co-sedimentations each sample contained 15 or 20μM of the N-terminus (*in isolation of the ABD) *while the actin concentration in each sample varied. After mixing the actin and peptide the samples were incubated at room temperature for 30 minutes before being spun down allowing the actin (and any peptide that bound to the actin) in each sample to form a pellet. The supernatant from the samples were run on a gel and the intensities of the peptide bands were recorded.*

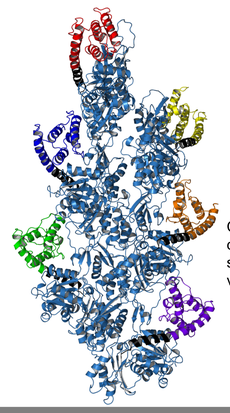
Data Analysis *- In Microsoft Excel the data from the cosedimentations was analyzed by comparing the peptide concentrations to a standard curve and by using the following fit equation*

$$\frac{B_{max} * [Actin]}{K_d + [Actin]}$$

These fitting parameters along with using Solver in Excel to minimize the sum of square errors between the data and the fit provided the maximal binding capacity (B_{max}) and binding affinity (Kd).

Computer Simulation *- NAMD with the force field CHARMM36 was used to collect data about the N-terminus in an implicit solvent. The software VMD (Visual Molecular Dynamics) was used to analyze this data and determine secondary structure probabilities for each residue in the sequence.*

Structural Hypotheses



β-III-spectrin Bound to Actin

My hypotheses are that the N-terminus contributes significantly to the actin-spectrin interaction and that when the N-terminus is not bound to actin it is disordered.

Cryo-EM based structural model for β-III-spectrin ABD (multiple colors) bound to actin (light blue). The N-terminus for each β-III-spectrin is denoted in black. Note that the CH2 domain is not visible since it is disordered.

N-terminus sequence: N-
STLSPTDFDSLEIQGQYSDINNRWDLPD
SDWDNDSSSARLFERSRIKALA-ABD

Results

Co-sedimentation of β-III-Spectrin IDR

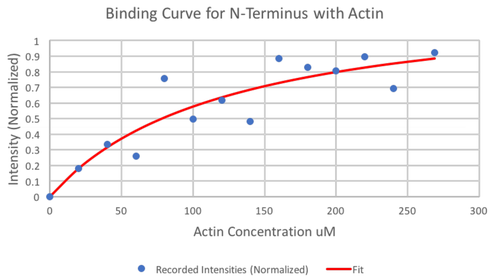


Figure 1. Binding curve from 9/11/17 for the N-terminus of β-III-spectrin. Each sample initially had a peptide concentration of 15*μM.* An equilibrium dissociation constant of 124*μM* was found.

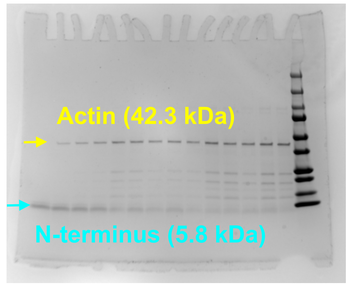
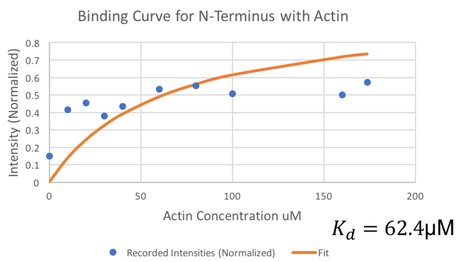


Figure 2. Gel from 9/11/17 co-sedimentation. Each sample initially contained 15*μM* of the peptide. It can be observed that the concentration of the peptide decreased while the actin concentration was increased. However, given how faint the bands became it was decided to increase the initial concentration of the peptide to 20*μM.*



$K_d = 62.4\mu M$

Figure 3. Binding curve from 10/16/17. In an attempt to optimize the results a standard curve using varying concentrations of the peptide was run on a separate gel. This curve was used to directly relate the intensity of the peptide band to the peptide concentration. The ten samples were also run across two gels allowing for a space between each sample.

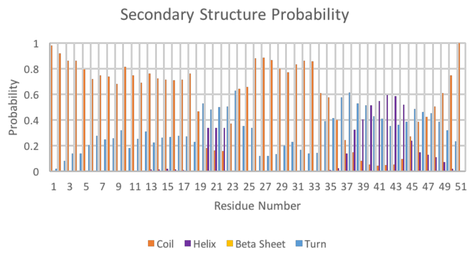


Figure 4. Secondary structure probability for each residue in the N-terminus. Probabilities are determined from 110ns of simulated time using the computer program NAMD. The probability for becoming helical was calculated by adding the probability for becoming an alpha and 3-10 helix.

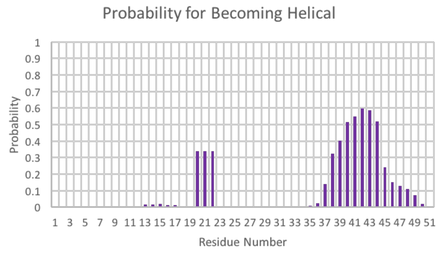


Figure 5. Highlighting helical probability specifically for each residue in the N-terminus. Probabilities are determined from 110ns of simulated time using the computer program NAMD.

Results Continued

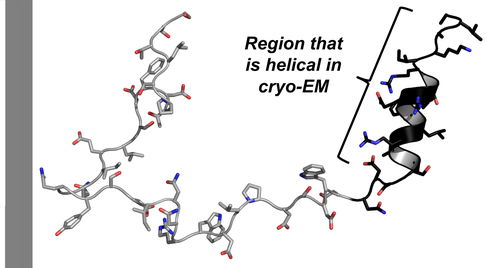
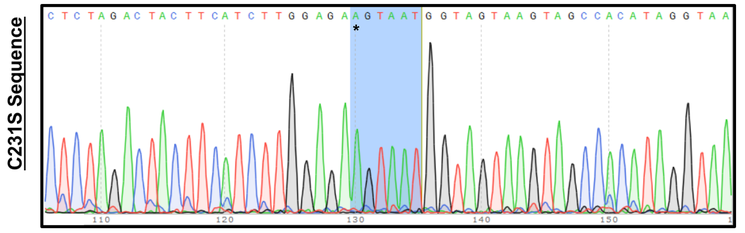


Figure 7. Conformation of the β-III-spectrin N-terminus derived from simulation. Region that is helical in modeled cryo-EM structure does have some helix structure in the absence of actin (black residues).



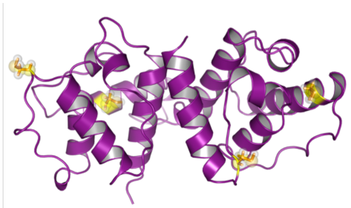


Figure 8. Sequencing results for the C231S mutation. The asterisk indicates the nucleotide that was changed (thymine to adenine).

Figure 9. Cysteines located in the actin binding domain of β-III-spectrin.

Summary

1. Using actin co-sedimentation experiments it was determined that the N-terminus of β-III-spectrin binds to actin in isolation of the ABD.
2. Possible N-terminus secondary structures are being simulated in an attempt to help visualize how it would contribute to the actin-spectrin interaction.
3. From these simulations it appears that the N-terminus may not be completely disordered when it is not in the presence of actin.
4. EPR will be used to determine if the N-terminus forms a stable conformer when bound to actin once the proper mutations are made.
5. Ultimately, these results will help us figure out a way to partially block the mutated β-III-spectrin[2] to attenuate its pathologically high actin affinity.

References

1. Avery, A. W. et al. Structural basis for high-affinity actin binding revealed by a β-III-spectrin SCA5 missense mutation. *Nature Communications8,* 1350.
2. Avery, A. W., Crain, J., Thomas, D. D., and Hays, T. S. (2016) A human β-III-spectrin spinocerebellar ataxia type 5 mutation causes high-affinity F-actin binding. *Scientific Reports6*, 21375.

Acknowledgements

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Biophysical Technology Center